FEBS Letters 583 (2009) 1257–1260

FEBS Letters

journal homepage: www.FEBSLetters.org

Fusion protein of Δ27LFn and EFn has the potential as a novel anthrax toxin inhibitor

Yirong Kong [1], Qiang Guo [1], Changming Yu, Dayong Dong, Jian Zhao, Chenguang Cai, Lihua Hou, Xiaohong Song, Ling Fu, Junjie Xu *, Wei Chen *

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China

ARTICLE INFO

Article history:
Received 9 February 2009
Revised 23 March 2009
Accepted 23 March 2009
Available online 28 March 2009

Edited by Renee Tsolis

Keywords:
Anthrax toxin
PA-binding domain of LF
PA-binding domain of EF
Toxin inhibitor
Fusion protein

ABSTRACT

PA-binding domain of LF (LFn) or PA-binding domain of EF (EFn) is the anthrax protective antigen (PA) binding domain of anthrax lethal factor (LF) or edema factor (EF). Here we show the development of a novel anthrax toxin inhibitor, fusion protein of N-terminal 27 amino acids deletion of LFn (Δ27LFn) and EFn. In a cell model of intoxication, fusion protein of Δ27LFn and EFn (Δ27LFn–EFn) was a 62-fold more potent toxin inhibitor than LFn or EFn, and this increased activity corresponded to a 39-fold higher PA-binding affinity by Biacore analysis. More importantly, Δ27LFn–EFn could protect the highly susceptible Fischer 344 rats from anthrax lethal toxin challenge. This work suggested that Δ27LFn–EFn has the potential as a candidate therapeutic agent against anthrax.

Structured summary:
MINT-7014735, MINT-7014747, MINT-7014761: *PA63* (uniprotkb:P13423) and *LF* (uniprotkb:P15917) *bind* (MI:0407) by *surface plasmon resonance* (MI:0107)

1. Introduction

Bacillus anthracis, the causative agent of anthrax infection, secretes two toxins which are comprised of three proteins: protective antigen (PA), a pore-forming protein; lethal factor (LF), a Zn-dependent endopeptidase that cleaves some members of the MAPKK family of intracellular signaling proteins; and edema factor (EF), a calmodulin-activated adenylate cyclase that increases cAMP concentration of eukaryotic cells [1]. It is believed that anthrax toxins play a role in all stages of anthrax infection. At the late stage of infection, even antibiotics have been administered, the infection can still be lethal because of the accumulation of the toxins. Logically, an effective therapeutic approach would include simultaneous killing of *B. anthracis* by antibiotics and inhibition of anthrax toxin with toxin inhibitors.

To exert its lethal effect, anthrax toxin must enter inside the host cell. PA (83 kDa) binds to specific cellular receptors/tumor endothelial marker 8 (TEM8) or capillary morphogenesis protein 2 (CMG2) [2,3] and is digested by a furin-like protease of the host cell into two fragments, PA20 and PA63, the latter heptamerizes to form a receptor-bound prepore and binds both LF and EF. The toxin–receptor complex is then internalized by receptor-mediated endocytosis and trafficked to a low-pH endosome where acidic conditions induce conversion of the prepore to a pore, and EF and LF are liberated into the cytosol by moving through the pore. PA-binding domain of LF (LFn) or PA-binding domain of EF (EFn) is the PA63 binding domain of LF or EF. They share 50% sequence similarity with each other. LFn was sufficient for binding PA63 and could act as a carrier for delivery of heterologous proteins across membranes in the presence of PA [4].

Several studies on anthrax toxin inhibitors have been published in recent years. These include dominant-negative mutants of PA [5], toxin-neutralizing antibodies [6,7], soluble receptors [8], and small molecular inhibitors of LF, EF [9,10]. Here we described the construction of a novel anthrax toxin inhibitor, genetically fused Δ27LFn (N-terminal 27 amino acids deletion of LFn) and EFn, and presented in vitro and in vivo studies that indicate this fusion protein has potential as a novel therapeutic agent against anthrax.

Abbreviations: PA, protective antigen; LF, lethal factor; EF, edema factor; LFn, PA-binding domain of LF; EFn, PA-binding domain of EF; Δ27LFn, N-terminal 27 amino acids deletion of LFn; Δ27LFn–EFn, fusion protein of Δ27LFn and EFn

* Corresponding authors. Address: Department of Applied Molecular Biology, Beijing Institute of Microbiology and Epidemiology, 20 Dongdajie, Fengtai, Beijing 100071, China. Fax: +86 10 63815273.
E-mail addresses: xujunjie1@gmail.com (J. Xu), cw789661@yahoo.com (W. Chen).

[1] These authors contributed equally to this article.

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.03.053

2. Materials and methods

2.1. Construction of plasmids

Plasmids and primers used in the study are listed in Table 1. Primers LF5 and LFn3 were used to amplify the coding sequence of LFn (aa 1–263 of LF) from *B. anthracis*; Primers Δ27LFn and LFn3 were used to amplify the coding sequence of Δ27LFn (aa 28–263 of LF); Primers EF5 and EFn3 were used to amplify the coding sequence of EFn (aa 1–254 of EF). Purified PCR products of LFn, Δ27LFn and EFn were cut with NdeI and XhoI and cloned into pET21a (Novagen), respectively, resulting in plasmids pET21a-LFn, pET21a-Δ27LFn and pET21a-EFn. A two-step procedure was used to construct expression vectors of fusion protein of Δ27LFn and EF (Δ27LFn–EF) and fusion protein of Δ27LFn and EF (Δ27LFn–EF). First, primers Δ27LFn and LFn-Linker3 (containing a (Gly4Ser)3 linker) were used to amplify the coding sequence of Δ27LFn-Linker which then were cloned into pET21a using 5′NdeI and 3′BamHI restriction sites, resulting in plasmid pET21a-Δ27LFn-Linker. Then, coding sequence of EFn and EF were amplified using primer pairs EF-Fusion5/EFn3 and EF-Fusion5/EF3, respectively, and were cloned into pET21a-Δ27LFn-Linker using 5′BamHI and 3′XhoI restriction sites. The two recombinant plasmids were named as pET21a-Δ27LFn-EFn and pET21a-Δ27LFn-EF. The same procedure was used to construct the expression vector of fusion protein of LFn and EF (LFn–EF). First, primers LFn5 and LFn-Linker3 were used to amplify the coding sequence of LFn-Linker which then was cloned into pET21a, resulting in plasmid pET21a-LFn-Linker. Then, coding sequence of EF was amplified with primer pairs EF-Fusion5/EF3, and was cloned into pET21a-LFn-Linker. The final plasmid was named as pET21a-LFn-EF.

2.2. Preparation of proteins

Escherichia coli strain BL21 (DE3) transformed by plasmids pET21a-LFn, pET21a-EFn, pET21a-Δ27LFn, pET21a-Δ27LFn-EFn, pET21a-Δ27LFn-EF, or pET21a-LFn-EF, respectively, was grown in LB broth at 37 °C until OD_{600} was between 0.6 and 0.7. Then, IPTG (0.5 mmol/L) was added to the culture to induce the expression of recombinant proteins at 28 °C for 3 h. The bacteria pellet was harvested by centrifugation, resuspended in the 20 mmol/L Tris buffer (pH 9.0), and lysed by sonication in ice bath. The insoluble debris in the lysate was removed by centrifugation and the clear supernatant was collected for the purification. The Ni-chelating column (GE Healthcare) was equilibrated with buffer (20 mmol/L Tris, 50 mmol/L imidazole and 0.5 mol/L NaCl, pH 9.0). The clarified sample was applied to the column and the target protein was eluted with a linear gradient from 50 mmol/L to 500 mmol/L imidazole. The proteins were further purified using a Superdex75 gel filtration column (GE Healthcare) with buffer PBS. The concentration of the purified proteins was determined using a BCA kit (PIERCE), and the purity of the proteins was examined by SDS–PAGE with coomassie blue staining.

2.3. Biomolecular interaction analysis (Biacore)

PA63 was prepared by trypsinization of PA [11]. Briefly, purified recombinant PA was incubated with trypsin (0.2 μg/ml) for 30 min at 25 °C in 25 mM HEPES, 1 mM $CaCl_2$, 0.5 M EDTA, pH 7.5. The reaction was stopped by addition of PMSF to 1 mM. Superdex75 was employed to separate PA63 and PA20.

All binding-kinetics experiments were performed using a BIAcore 3000 instrument (GE Healthcare). PA63 was covalently linked to the CM5 chip. Concentrations of different recombinant proteins ranged from 0 nmol/L to 200 nmol/L in HBS-EP buffer (pH 7.4). Serial injections were made at 20 μl/min at 25 °C. To calculate the binding constants, the data obtained were analyzed by BIAevaluation software version 4.0. Results were the average of two independent experiments.

2.4. In vitro intoxication experiments

J774A.1 cells intoxication experiments were performed with a modified protocol described before [11]. Briefly, Murine macrophage-like J774A.1 cells were grown to confluence in wells of a 96-well plate. Then aliquots of 100 μl of medium containing PA (100 ng/ml), LF (100 ng/ml) and various concentrations of fusion proteins were added to the cells. After a 3-h incubation at 37 °C, cell viability was assessed by MTT staining.

2.5. Anthrax lethal toxin challenge experiments in rats

Rats challenge experiments were approved by the Animal Care and Use Committee of our Institute. Male Fischer 344 rats (3/group) were injected intravenously 300 μl/rat of lethal toxin (40 μg of PA plus 10 μg of LF in PBS), or lethal toxin plus Δ27LFn-EFn (213 μg or 426 μg per rat). Rats were monitored continuously for symptoms of intoxication and death for 48 h.

Table 1
Plasmids and primers used in the study.

Plasmids or primers	Phenotype or sequence	Resource
Plasmids		
pET21a	Expression vector containing 6×His tag	
pET21a-LFn	Vector for expression of LFn (aa 1–263 of LF), derivative of pET21a	This study
pET21a-EFn	Vector for expression of EFn (aa 1–254 of EF), derivative of pET21a	This study
pET21a-Δ27LFn	Vector for expression of Δ27LFn (aa 28–263 of LF), derivative of pET21a	This study
pET21a-Δ27LFn-Linker	Vector containing Δ27LFn and a $(G_4S)_3$ Linker, derivative of pET21a	This study
pET21a-Δ27LFn-EFn	Vector for expression of Δ27LFn–EFn, derivative of pET21a-Δ27LFn-Linker	This study
pET21a-Δ27LFn-EF	Vector for expression of Δ27LFn–EF, derivative of pET21a-Δ27LFn-Linker	This study
pET21a- LFn-Linker	Vector containing LFn and a $(G_4S)_3$ Linker, derivative of pET21a	This study
pET21a- LFn-EF	Vector for expression of LFn–EF, derivative of pET21a-LFn-Linker	This study
Primers		
LF5	aaccatatggcgggcggtcatggtgatgta	
LFn3	aacctcgagccgttgatctttaagttcttc	
Δ27LF5	aaccatatgcgaaataaaacacaggaagagc	
EF5	aaccatatgaatggacattacactgagagtgatattaaaag	
EF3	aacctcgagtttttcatcaataatttttgc	
EFn3	aacctcgagaccttctttcttcaaactttcacttattttc	
EF-Fusion5	aacggatccatgaatgaacattacactgagagtgatattaaaag	
LFn-Linker3	aagggatccagagcccccgccaccagagcccccgccaccagagcccccgccaccccgttgatctttaagttcttc	

2.6. Analysis of cellular cAMP

Recombinant EF was prepared as described before [12]. To measure cAMP accumulation inside cells, CHO-K1 cells were seeded at 1×10^4 cells per well in a 96-well plate. One day later, cells were incubated with various concentrations of recombinant proteins and PA (1 μg/ml) for 2 h. After removal of the culture medium, the levels of cAMP were determined by enzyme immunoassay following the manufacturer's instruction (The direct cAMP assay kit, nonacetylated version, GE Healthcare).

3. Results and discussion

3.1. Expression and purification of recombinant proteins

All recombinant proteins were produced in the cytoplasm of recombinant *E. coli* strains. They were all fused with a 6×His tag and could be purified with a Ni-chelating column. After an additional size-exclusion chromatography step, LFn, EF, Δ27LFn, Δ27LFn–EFn, LFn–EF and Δ27LFn–EF were obtained in high purity as judged by SDS–PAGE (Fig. 1).

3.2. Fusion protein Δ27LFn–EFn had higher toxin neutralization activity than LFn, EFn or Δ27LFn due to higher PA63 affinity

LFn or EFn is the PA-binding domain of LF or EF. To determine whether LFn and EFn have the potential as anthrax toxin inhibitors, they were tested for the capacity to inhibit lethal toxin (LF plus PA) induced cytotoxicity in J774A.1 cells. A concentration of 323 nmol/L LFn or EFn (molar ratio was around 300:1 to PA) was needed to obtain complete protection of cells (Fig. 2). The low potency of LFn and EFn suggested that they might have low binding affinity to PA63. We then used Biacore to compare the binding kinetics of LFn or LF to PA63. The observed KA value of LFn was 6.26×10^7 M^{-1}, 6.6-fold lower, compared with that of LF (Table 2). Therefore, the low potency of LFn correlates with the low binding affinity of LFn to PA63.

A maximum of three molecules of LF or EF can bind simultaneously per PA63 heptamer [13]. Deleting 27 or 36 residues of LFn strongly inhibited acid-triggered translocation of LFn across the plasma membrane of CHO-K1 cells without significantly affecting binding to PA63 [14]. We hypothesized Δ27LFn, a 27 residues deleting form of LFn, could be more potent than LFn if one Δ27LFn could influence the translocation of two LF or EF by destroying PA63 channel or other unknown way. As shown in Fig. 2, a concentration of 81 nmol/L Δ27LFn (molar ratio was around 75:1 to PA) was sufficient to obtain complete protection of cells, which meant Δ27LFn was about 4-fold more potent than LFn. In binding-kinetics study, the KA value of Δ27LFn was 1.58×10^8 M^{-1}, 2.5-fold higher than that of LFn (Table 2), which suggests the 27 amino acids deletion might enhance the affinity of LFn to PA63.

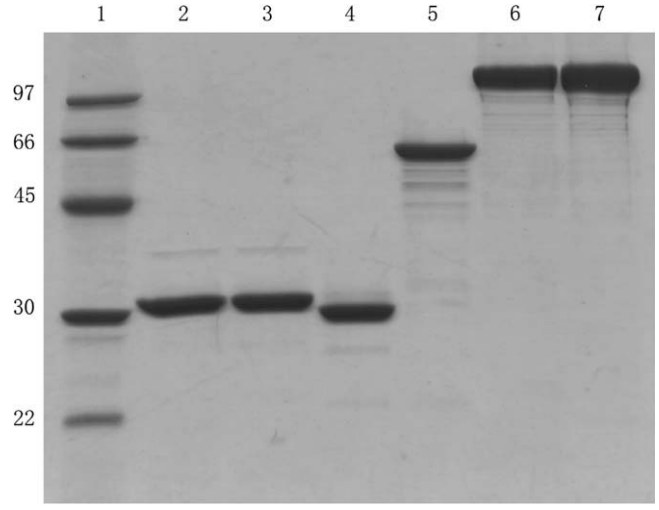

Fig. 1. Analysis of purified LFn, EFn, Δ27LFn, Δ27LFn–EFn, LFn–EF and Δ27LFn–EF by SDS–PAGE. Lane 1, protein markers; lane 2, LFn (~31 kDa); lane 3, EFn (~31 kDa); lane 4, Δ27LFn (~29 kDa); lane 5, Δ27LFn–EFn (~60 kDa); lane 6, LFn–EF (~120 kDa); and lane 7, Δ27LFn–EF (~119 kDa).

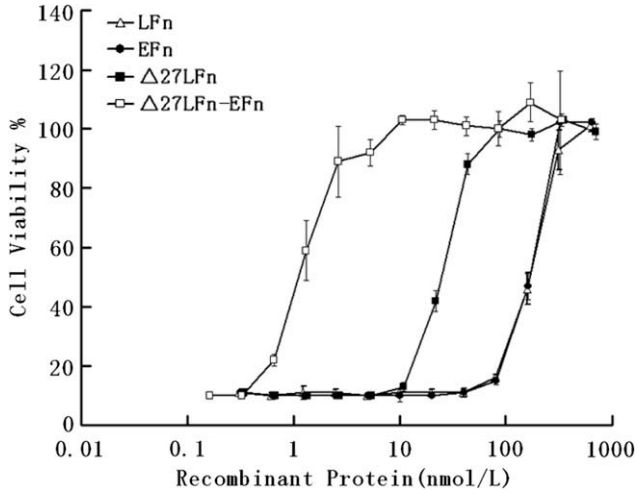

Fig. 2. In vitro intoxication assay. LFn, EFn, Δ27LFn and Δ27LFn–EFn were tested for their ability to protect cells from intoxication by incubating J774A.1 cells with 100 ng/ml PA and 100 ng/ml LF mixed with varying amounts of proteins respectively. Cell viability was then assessed 3 h later by MTT assay. Data points represent the mean cell viability% values for triplicate samples.

It was reported that cross-linked LFn was a more potent anthrax toxin inhibitor than LFn monomer [15]. We hypothesized fusion protein of Δ27LFn and EFn could be better than Δ27LFn monomer. To test this hypothesis, Δ27LFn–EFn was constructed and tested. A concentration of 5.2 nmol/L Δ27LFn–EFn (molar ratio was around 5:1 to PA) was sufficient to obtain complete protection (Fig. 2), which meant Δ27LFn–EFn was 62-fold more potent than LFn and about 15-fold more potent than Δ27LFn. The KA value of Δ27LFn–EFn was 2.42×10^9 M^{-1}, 39-fold higher compared with that of LFn, and 15-fold higher compared with that of Δ27LFn. So the higher toxin neutralization activity of Δ27LFn-EFn correlates with its higher affinity to PA63.

3.3. Δ27LFn–EFn protected the rats against anthrax lethal toxin challenge

The anti-toxin efficacy of Δ27LFn–EFn was further evaluated in Fischer 344 rats. Control rats injected with 10 μg of LF plus 40 μg of PA died in about 65 min, while the rats receiving toxin and 426 μg Δ27LFn–EFn (molar ratio was 16:1 to PA) were fully protected and showed no signs of intoxication symptoms. A 2-fold lower concentration of Δ27LFn–EFn (213 μg, molar ratio was 8:1 to PA) was not effective in protecting the rats, although it did extend their survival significantly, to an average of 200 min, compared with the rats receiving the toxin alone (Table 3).

3.4. Translocation of Δ27LFn–EFn

The structure of Δ27LFn–EFn is different from that of LFn/EFn: it has two PA63 binding domains and its N terminus is truncated. To explore the effects of those difference on the translocation, we constructed two fusion proteins, LFn–EF and Δ27LFn–EF, and measured translocation activities of the constructs. In a cellular cAMP

Table 2
Kinetic parameters for binding of LF, EFn, Δ27LFn and Δ27LFn–EFn to PA63.

Protein	k_a $M^{-1}S^{-1}$ ± S.D.	k_d S^{-1} ± S.D.	K_A M^{-1}[a] ± S.D.	K_D M[a] ± S.D.
LF	$4.48 \times 10^5 \pm 1.63 \times 10^5$	$1.09 \times 10^{-3} \pm 1.41 \times 10^{-5}$	$4.12 \times 10^8 \pm 1.55 \times 10^8$	$2.62 \times 10^{-9} \pm 9.90 \times 10^{-10}$
LFn	$6.80 \times 10^5 \pm 7.14 \times 10^4$	$1.12 \times 10^{-2} \pm 3.37 \times 10^{-3}$	$6.26 \times 10^7 \pm 1.24 \times 10^7$	$1.63 \times 10^{-8} \pm 3.25 \times 10^{-9}$
Δ27LFn	$4.46 \times 10^5 \pm 1.15 \times 10^5$	$3.18 \times 10^{-3} \pm 1.05 \times 10^{-3}$	$1.58 \times 10^8 \pm 8.56 \times 10^7$	$7.56 \times 10^{-9} \pm 4.32 \times 10^{-9}$
Δ27LFn–EFn	$1.27 \times 10^6 \pm 2.33 \times 10^5$	$5.37 \times 10^{-4} \pm 7.64 \times 10^{-5}$	$2.42 \times 10^9 \pm 7.85 \times 10^8$	$4.37 \times 10^{-10} \pm 1.43 \times 10^{-10}$

[a] The equilibrium association/dissociation constant is calculated from kinetic measurements of the association and dissociation rate constants according to $K_A = k_a/k_d$ or $K_D = k_d/k_a$. Data represent the mean values for two independent experiments.

assay (Fig. 3), the level of EF-induced cAMP accumulation in CHO cells treated with PA plus LFn–EF appeared no obvious difference with cells treated with PA plus EF. But cytoplasm cAMP level in cells treated with PA plus Δ27LFn–EF was lower than cells treated with PA and EF. These results suggested N-terminal truncations of 27 residues did influence the translocation of Δ27LFn–EF while the two PA63 binding domains did not. We thought lower efficiency of translocation may be a merit of Δ27LFn–EFn as a therapeutic drug, for it may reduce the potential interference of the protein to the normal function of host cells.

By preventing intoxication in vivo with an inhibitor: PA molar ratio around 16:1, Δ27LFn–EFn seems less effective than soluble receptors (sCMG2) or toxin-neutralizing antibodies [6–8]. But Δ27LFn–EFn may have several advantages over them. First, Δ27LFn–EFn was over expressed in *E. coli* system which is the simple and cheap way to obtain large quantities of recombinant proteins. Second, PA antibodies would be non-effective against genetically modified strains of *B. anthracis* that express antigenically altered forms of PA, while the therapeutic function of Δ27LFn–EFn will not be affected as any altered forms of PA that retains function will, presumably, still bind to LF/EF. Third, sCMG2 may influence normal physiological functions of natural CMG2, while Δ27LFn–EFn may be safe as it does not have catalytic domain of anthrax toxin. In conclusion, the data presented here illustrate that Δ27LFn–EFn has the potential to act as a novel anthrax toxin inhibitor.

Table 3
Δ27LFn–EFn protected rats against anthrax lethal toxin challenge.

Treatment (molar ratio)[a]	No. of survivors/ total	Time to death mean ± S.D.	P[b]
LeTx only[c]	0/3	65 ± 3 min	
PBS[d]	3/3	>48 h[e]	
Δ27LFn–EFn (8:1)	0/3	272 ± 45 min	0.000652
Δ27LFn–EFn (16:1)	3/3	>48 h	

[a] Molar ratio refers to Δ27LFn–EFn: PA.
[b] For comparisons to the LeTx-only control group, by *t*-test.
[c] LeTx-only group (40 μg of PA plus 10 μg of LF per rat).
[d] Negative control group receiving PBS and no LeTx.
[e] Rats were monitored >48 h.

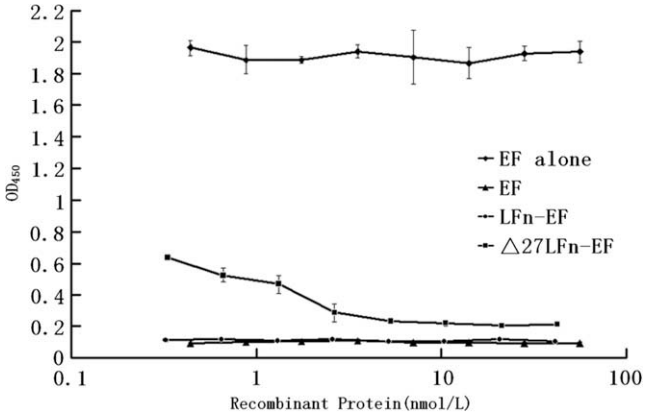

Fig. 3. Analysis of cellular cAMP. CHO-K1 cells were treated with EF alone, EF + PA, LFn–EF + PA, or Δ27LFn–EF + PAm respectively. A fixed PA concentration, 1 μg/ml and a series of 2-fold dilution of EF, LFn–EF, and Δ27LFn–EF were used. A competition ELISA was used to detect the cAMP level in cell cytoplasm. Higher OD_{450} value represents lower cAMP level in cell cytoplasm. Data points represent the mean OD_{450} values for triplicate samples.

Acknowledgments

We thank Drs. Xiaowei Zhou and Long Xu at Beijing Institute of Biotechnology for helping Biacore experiments. This work was supported by National Natural Science Foundation of China (30300016 and 30571745) and National High Technology Research and Development Program of China (2006AA02A232).

References

[1] Collier, R.J. and Young, J.A. (2003) Anthrax toxin. Annu. Rev. Cell Dev. Biol. 19, 45–70.
[2] Bradley, K.A., Mogridge, J., Mourez, M., Collier, R.J. and Young, J.A. (2001) Identification of the cellular receptor for anthrax toxin. Nature 414, 225–229.
[3] Scobie, H.M., Rainey, G.J., Bradley, K.A. and Young, J.A. (2003) Human capillary morphogenesis protein 2 functions as an anthrax toxin receptor. Proc. Natl. Acad. Sci. USA 100, 5170–5174.
[4] Arora, N. and Leppla, S.H. (1993) Residues 1–254 of anthrax toxin lethal factor are sufficient to cause cellular uptake of fused polypeptides. J. Biol. Chem. 268, 3334–3341.
[5] Sellman, B.R., Mourez, M. and Collier, R.J. (2001) Dominant-negative mutants of anthrax toxin subunit: an approach to therapy of anthrax. Science 292, 695–697.
[6] Albrecht, M.T., Li, H., Williamson, E.D., et al. (2007) Human monoclonal antibodies against anthrax lethal factor and protective antigen act independently to protect against *Bacillus anthracis* infection and enhance endogenous immunity to anthrax. Infect. Immun. 75, 5425–5433.
[7] Wild, M.A., Xin, H., Maruyama, T., Nolan, M.J., Calveley, P.M., Malone, J.D., Wallace, M.R. and Bowdish, K.S. (2003) Human antibodies from immunized donors are protective against anthrax toxin in vivo. Nat. Biotechnol. 21, 1305–1306.
[8] Scobie, H.M., Thomas, D., Marlett, J.M., Destito, G., Wigelsworth, D.J., Collier, R.J., Young, J.A. and Manchester, M. (2005) Soluble receptor decoy protects rats against anthrax lethal toxin challenge. J. Infect. Dis. 192, 1047–1051.
[9] Shoop, W.L., Xiong, Y., Wiltsie, J., et al. (2005) Anthrax lethal factor inhibition. Proc. Natl. Acad. Sci. USA 102, 7958–7963.
[10] Shen, Y., Zhukovskaya, N.L., Zimmer, M.I., et al. (2004) Selective inhibition of anthrax edema factor by adefovir, a drug for chronichepatitis B virus infection. Proc. Natl. Acad. Sci. USA 101, 3242–3247.
[11] Zhang, J., Xu, J., Li, G., Dong, D., Song, X., Guo, Q., Zhao, J., Fu, L. and Chen, W. (2006) The 2β2–2β3 loop of anthrax protective antigen contains a dominant neutralizing epitope. Biochem. Biophys. Res. Commun. 341, 1164–1171.
[12] Dong, D., Xu, J., Song, X., Fu, L. and Chen, W. (2005) Expression and analysis of biological activity of the recombination anthrax edema factor. Acta Microbiol. Sin. 45, 459–462.
[13] Cunningham, K., Lacy, D.B., Mogridge, J. and Collier, R.J. (2002) Mapping the lethal factor and edema factor binding sites on oligomeric anthrax protective antigen. Proc. Natl. Acad. Sci. USA 99, 7049–7053.
[14] Zhang, S., Finkelstein, A. and Collier, R.J. (2004) Evidence that translocation of anthrax toxin's lethal factor is initiated by entry of its N terminus into the protective antigen channel. Proc. Natl. Acad. Sci. USA 101, 16756–16761.
[15] Juris, S.J., Melnyk, R.A., Bolcome, R.E., Chan, J. and Collier, R.J. (2007) Cross-linked forms of the isolated N-terminal domain of the lethal factor are potent inhibitors of anthrax toxin. Infect. Immun. 75, 5052–5058.